Probiotics in the Space Food System: Delivery, Microgravity Effects, and the Potential Benefit to Crew Health

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As mission distance and duration increase, the need grows for non-invasive disease prevention and immunomodulation, especially given the limited medical response capability expected for these missions and the immune dysregulation documented in crew. Additionally, changes in diet, lifestyle, antibiotic usage, and the environmental stresses during spaceflight may alter crewmembers' intestinal microbiome. The addition of probiotic bacteria to the space food system is expected to confer immunostimulatory benefits on crewmembers, with the potential to counteract the immune dysregulation that has been documented in spaceflight. Based on previous studies that demonstrated unique microbiological responses to the low shear environment of spaceflight, probiotic organisms hold the potential to induce enhanced beneficial responses through mechanisms, such as beneficial interactions with human immune cells and repression of colonization of pathogens on the mucosa. The work presented here will begin to address two research gaps related to providing probiotics in spaceflight: 1) delivery, and 2) the effect of the low shear microgravity environment on probiotic attributes. The probiotic *Lactobacillus acidophilus* was selected for investigation due to its wide commercial use and documented benefits that include inhibition of virulence related gene expression in pathogens and mucosal stimulation of immune cells.

The delivery system for probiotics has not been determined for spaceflight, where the food system is shelf stable and the lack of refrigeration prevents the use of traditional dairy delivery methods. In order to demonstrate the potential of the space food system to deliver viable probiotic bacteria to crewmembers, the probiotic *L. acidophilus* was packaged in high barrier flight packaging in nonfat dry milk (NFDM) or retained in commercial capsule form. Viable cells were enumerated over 8 months of storage at 22, 4, and -80ºC. The survival of *L. acidophilus* rehydrated in NFDM, in a PBS control, and directly from the capsule was also evaluated following stress challenge with simulated gastric and intestinal juices to determine the method that would deliver the most viable cells to the intestine, where they would be expected to confer beneficial effects. *L. acidophilus* was found to be stable to gastric and intestinal juice challenge when delivered in rehydrated NFDM, even after two hours of exposure. In comparison, *L. acidophilus* was reduced by 1-5 logs when exposed to gastric and intestinal juice directly and when rehydrated in a PBS control. Shelf life data indicated that *L. acidophilus* would require refrigerated or frozen storage to remain viable at adequate levels over the multi-year storage periods required for spaceflight. This study suggests that the protective effect provided by the dairy matrix, and not merely rehydration prior to consumption, extends probiotic viability and stress tolerance during storage in spaceflight and in simulated digestion conditions more adequately than a capsule.

In addition to effective delivery, it is essential to understand the microgravity effects on the stress tolerances and genetic expression of probiotic bacteria to enable optimization of growth, survival, strain selection, and conferred benefits in spaceflight. In our current study, ground-based characterization of the growth, stress response, and transcriptomic response of *L. acidophilus* will be accomplished using the low shear modeled microgravity (LSMMG) culture environment in the rotating wall vessel (RWV). We hypothesize that *L. acidophilus* will maintain or improve its growth and stress response following culture in the LSMMG environment, and that transcriptomic analysis will define the associated molecular mechanism(s), resulting in the ability to optimize strain selection.